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A Goal of microbiological research has been the development of methods for the detection and enumeration of pathogenic bacterial species in solid waste and waste effluents. Attempts to isolate such organisms from solid waste on a routine basis have not been fruitful due to low initial numbers and/or to relatively short periods of survival. Pathogenic microoganisms in waste are constantly subjected to such debilitating environmental factors as chemical additives, drying, freezing, heat, and pH extremes. These factors often affect cultivation of these organisms in media originally designed for diagnostic purposes. For these reasons attention was directed primarily toward the development of methods for the detection and enumeration of a group of organisms of sanitary and health significance.

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METHODS FOR BACTERIOLOGICAL EXAMINATION OF SOLID WASTE AND WASTE EFFLUENTS

U. S. ENVIRONMENTAL PROTECTION AGENCY

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SECTION A. Introduction and General Laboratory Procedures

1. INTRODUCTION

1.1 A goal of microbiological research has been the development of methods for the detection and enumeration of pathogenic bacterial species in solid waste and waste effluents. Attempts to isolate such organisms from solid waste on a routine basis have not been fruitful due to low initial numbers and/or to relatively short periods of survival. Pathogenic microoganisms in waste are constantly subjected to such debilitating environmental factors as chemical additives, drying, freezing, heat, and pH extremes. These factors often affect cultivation of these organisms in media originally designed for diagnostic purposes. For these reasons attention was directed primarily toward the development of methods for the detection and enumeration of a group of organisms of sanitary and health significance. Three procedural lines of investigation were undertaken:

1.1.1 To develop methods suitable for indicating the sanitary quality of solid waste before and after processing or disposal.

1.1.2 To develop methods suitable for determining the efficacy of operational procedures in removing or destroying the microorganisms.

1.1.3 To develop methods suitable for indicating the health hazard of solid waste in which pathogenic species may be present in small numbers.

1.2 An investigation was made to evaluate presently employed bacteriological methods applicable to solid waste and related materials.

This evaluation led to the establishment of reliable methods which are best suited to routinely measure under practical conditions the bacteriological quality of solid waste, incinerator residue, industrial and quench waters, leachate, stack emissions, and dust in and around waste processing areas. The methods described here will determine:

1.2.1 Total viable bacterial cell number

1.2.2 Total coliforms

1.2.3 Fecal coliforms

1.2.4 Heat-resistant spores

1.2.5 Enteric pathogens, especially Salmonella sp.

It should be remembered that minor changes in technical procedure may result in marked changes in the validity of the data.

2. GENERAL LABORATORY PROCEDURES

2.1 Glassware washing

2.1.1 All glassware known to contain infectious material must be sterilized by autoclaving before washing.

2.1.2 All glassware which is to be used in microbiological tests must be thoroughly washed before sterilization using a suitable detergent and hot water, followed by hot water and distilled water rinses. Six to twelve rinses may be required to remove all traces of inhibitory residues from the glass surface.

2.2 Sterilization

2.2.1 Dry heat is utilized for the sterilization of glass sampling bottles, foil covered flasks, beakers, graduates, pipets packed tightly

in sealed cans, or articles which are corrosively attacked by steam. Recommended time-temperature ratio for dry heat sterilization is 170 C for 2 hr.

2.2.2 Saturated steam under pressure (or autoclaving) is the most frequently used sterilization method. Media, dilution water, and materials (such as rubber, paper, cotton, cork, heat-stable plastic tubes and closures) are sterilized by autoclaving at 121 C. Sterilization time for media and dilution water for volumes up to 500 ml is 15 min; 1000 ml quantities are held for 20 min.; instruments - 15 min; gloves - 20 min.; and packs - 30 min., measured from the time the autoclave temperature reaches 121 C.

2.2.3 Membrane filters are sterilized for 10 min at 121 C with fast steam exaust at the end of the sterilization process.

2.2.4 Heat-sensitive carbohydrates and other compounds should be sterilized by passage through a cellulose esther membrane or another bacteria retaining filter.

2.3 Culture Media

2.3.1 The use of dehydrated media is recommended whenever possible, as these products offer the advantages of good consistency from lot to lot, requires less labor in preparation, and are more economical. Each lot should be tested for performance before use.

2.3.2 Measurement of the final pH of a prepared culture medium should be accomplished colorimetrically or potentiometrically after autoclaving and cooling. Acceptable pH range is 7.0 ± 0.1.

2.3.3 Media should be stored in a cool, dry, and dark place to avoid dehydration, deterioration, and adverse light affect. Storage in the refrigerator usually prolongs the shelf life of most media. Media should not be subjected to long periods of storage because certain chemical reactions may occur in a medium even at refrigerator temperatures.

2.3.4 Many of the media referred to in Methods can be obtained from commerical sources in a dehydrated form with complete information on their preparation, therefore these media will be listed but not described in this section. Described in this section are those media which are formulated from ingredients or from dehydrated materials.

2.3.5 Tabulation of culture media

Bacto-agar

Bismuth sulfite agar

Blood agar

Brain heart infusion broth

Brilliant green lactose bile, 2%

Brilliant green agar

Coagulase mannitol agar

Dextrose

E. C. broth

Eosin methylene blue agar, Levine

Fluid thioglycollate medium

Gelatin

H-broth

Indole nitrite medium

KCN medium

Lactose

Lactose tryptose broth

Lauryl tryptose broth

Lysine decarboxylase medium

M-Endo broth

M-FC broth

MacConkey agar

Malonate broth, Ewing modified

Maltose

Mannitol salt agar

Mannitol

Methyl red-Voges Proskauer medium

Nitrate broth

Nutrient agar

Phenol red broth base

Phosphate buffer, APHA, pH 7.2

Sabouraud dextrose agar

Salmonella Shigella agar

SBG enrichment broth

Selenite-F enrichment broth

SIM medium

Simmons citrate agar

Sucrose

Triple sugar iron agar

Trypticase soy agar

Tryptone glucose extract agar

Urea agar base concentrate (sterile)

X_LD agar

2.3.6 Culture media requiring preparation

2.3.6.1 Blood Agar -- Suspend 40 gms of trypticase soy agar in a liter of distilled water. Mix thoroughly. Heat with agitation and boil for one minute. After solution is accomplished, sterilize by autoclaving for 15 min at 121 C. Cool agar to 45 to 50 C, and add 5 to 7 per cent sterile defibrinated sheep blood mixing evenly throughout the medium. Pour into sterile Petri dishes. After solidification invert dishes and incubate overnite.

2.3.6.2 Phenol Red Broth Base -- Dissolve 15 gms in a liter of distilled water. Add five to ten gms of desired carbohydrate. Use Durham fermentation tubes for detection of gas formation. Arrange tubes loosely in suitable containers and sterilize to 116 to 118 C for 15 min.

2.3.6.3 Phosphate Buffer Solution -- To prepare stock phosphate buffer solution, dissolve 34.0 g potassium dihydrogen phosphate, KH_2PO_4 in 500 ml distilled water, adjust to pH 7.2 with IN NaOH, and dilute to 1 liter with distilled water.

Add 1.25 ml stock phosphate buffer solution to 1 liter distilled water. Dispense in amounts that will provide 99 ± 2.0 ml or 9 ± 0.2 ml after autoclaving.

SECTION B. Methods for Collection and Preparation of Samples

1. METHOD FOR COLLECTION OF SOLID WASTE OR SEMI-SOLID WASTE SAMPLES

1.1 Equipment and materials

1.1.1 Sample containers, specimen cups, sterile, 200-ml size (Falcon Plastics, Los Angeles)

1.1.2 Sampling tongs, sterile (stainless steel, angled tips, 18" long)

1.1.3 Shipping container, insulated, refrigerated, 6"x12" I.D.*

1.1.4 Disposable gloves

1.2 Procedure

1.2.1 Using sterile tongs collect 20 to 40 random 100 gm to 200 gm samples and place in sterile sampling containers. When collecting samples from contaminated sources wear disposable gloves and avoid contaminating the outside of the container.

1.2.2 Identify samples on tag and indicate time and date of sampling. If incinerator residue samples are taken, record operating temperatures of incinerator.

1.2.3 Deliver samples to laboratory. It is recommended that the examination be started preferably within 1 hr. after collection; the time elapsing between collection and examination should in no case exceed 30 hours. Sample temperature is maintained as near that at collection time as possible.

2. METHOD FOR COLLECTION OF LIQUID SAMPLES - QUENCH OR INDUSTRIAL WATERS OR LEACHATE.

2.1 Equipment and materials

* If sample is shipped to a laboratory for analysis and examination can not begin within one hour of collection, the container must be insulated and sample maintained below 10°C during the maximum of 6 hours. Such samples should be refrigerated upon receipt in the laboratory and processed within 2 hours.

2.1.1 Sample bottle, screw capped, 250 ml size, sterile, or plastic bag, 16-oz. size, sterile.

2.2 Procedure

2.2.1 Collect sample in bottle or plastic bag leaving an air space in the container to facilitate mixing of the sample prior to examination. When collecting samples from contaminated sources wear disposable gloves and avoid contaminating the outside of the container.

2.2.2 Identify and deliver samples to laboratory. When shipping of samples to laboratory, protect containers from crushing or other damage, and maintain temperature as near that at collection time as possible.* Examine within 4 hours. If water sample contains residual chlorine a dechlorinating agent such as sodium thiosulfate is added to collection bottles to neutralize any residual chlorine and to prevent a continuation of the bactericidal action of chlorine during the time the sample is in transit to the laboratory. The sodium thiosulfate is added to the clean sample bottle before sterilization in an amount to provide an approximate concentration of 100mg/ℓ in the sample.

3. METHOD FOR COLLECTION OF INCINERATOR STACK EFFLUENTS

3.1 Equipment and materials

3.1.1 Armstrong portable sampler (2), equipped with sampling assembly (Fig. 1). The sampler is mounted on a steel plate (6 by 12 inches) and can be enclosed by a metal cover with a handle attached (Fig. 1). On one side of the base is a vacuum pump with a 6-ft cord

and switch. The pump is capable of drawing up to 1 cu. ft. per min of air [vacuum of 5.6 inches (14.3 cm) of water]. On the other side of the base, a 700 ml wide-mouth, Pyrex bottle contains 300 ml of 0.067 M phosphate buffered solution, (pH 7.2), prepared by standard methods(4). The two-hole rubber stopper has a 1-inch (2.54 cm) piece of cotton-plugged glass tubing in one of the two holes. The stopper, glass tube, and contents of the bottle are maintained sterile. The bottle is held to the base plate by three removable spring clips, which are attached at the base and at a wire triangle slipped over the top of the bottle. The sampling probe is made of stainless-steel tubing of appropriate diameter [e.g., 0.25-inch inside diameter (0.64 cm)]. The probe end has a right-angle bend so that the opening faces the stack gas current. The tubing must be long enough to reach all parts of the stack. The tubing is coiled to permit additional cooling of the gases and is straight for 1 or 2 ft (30.48 or 60.96 cm) at a right angle to the other straight length. Before use, the sampling probe is sterilized by dry heat sterilization. It is important to keep the inside of the probe dry to minimize adsorption of micro-organisms on the walls of the tubing. When sampling, the probe is inserted into the stack at locations that will yield a representative sample. The other end of the sterile probe is inserted through the sterile rubber stopper to approximately 0.5 inch (1.27 cm) above the buffered water. This is done to reduce the frothing that would occur if the probe were inserted below the surface; enough froth results to capture the microorganisms.

3.2 Procedure

3.2.1 Draw stack effluent through the sterile stainless steel tube by a 1.0 cfm vacuum pump, cooling the tube by a water jacket.

3.2.2 Obtain a 10 cubic foot sample by drawing the stack effluent for 10 min.

3.2.3 Identify sample on tag and examine within 4 hours. The Armstrong portable sampler provides a method for qualitative, non-isokinetic sampling, adjustable to isokinetic conditions

4. METHOD FOR COLLECTION OF DUST SAMPLES

4.1 Equipment and materials

4.1.1 Andersen sampler (3)

4.1.2 Trypticase soy agar containing 5% sheep blood to trap skin and respiratory tract bacteria (6 plates per sample).

4.1.3 Eosin methylene blue agar to enumerate intestinal tract bacteria.

4.2 Procedure

4.2.1 Draw air through the sterile, assembled sampler at 1.0 cfm with a vacuum of 15 inches of mercury.

4.2.2 Remove agar plates from the sampler, cover, and incubate at 35±0.5 C. Use aseptic technique throughout the procedure.

5. METHOD FOR PREPARATION OF SOLID AND SEMI-SOLID SAMPLES FOR ANALYSES

5.1 Equipment and materials

5.1.1 Cold phosphate buffer, 0.067 M, (pH 7.2), sterile (4)

5.1.2 Blender, Waring (Model 1088), sterile

5.1.3 Balance, with weights, 500 gm capacity

5.1.4 Tongs, sterile

5.1.5 Beakers, two, 5000 ml and 1000 ml sizes, sterile, covered with aluminum foil before sterilization.

5.2 Procedure

5.2.1 Using aseptic technique composite all random samples into a 5000 ml beaker. Mix well.

5.2.2 Weigh 200 gm of the subsample into a 1000 ml beaker

5.2.3 Transfer the weighed sample to a sterile blender

5.2.4 Add 1800 ml of sterile phosphate buffered solution to the blender.

5.2.5 Homogenize for 15 sec. at 17,000 rpm [16]

5.2.6 Prepare a series of decimal dilutions as described below. [Section C 1.1 - 1.3]

Solid waste and residue samples for enteric pathogenic bacteria are examined directly without homogenization.

SECTION C. Bacteriological Examination of Waste and Related Materials (description of methods)

1. METHOD FOR PREPARATION OF DECIMAL DILUTIONS OF A SOLID, SEMI-SOLID OR LIQUID WASTE MATERIAL

1.1 Immediately after homogenization of any sample [Section B 5.2.1 - 5.2.6] transfer 1 ml portion of the homogenate (10^{-1} dil.) to a dilution bottle containing 99 ml of phosphate buffered solution. Stopper and shake the bottle 25 times.

1.2 Prepare dilutions as indicated in Figure 2.

1.3 Again shake each dilution vigorously 25 times after adding aliquot of sample.

These dilutions are used to inoculate a series of selected culture media for the detection of various groups of micro-organisms as described in the following sections of this manual.

2. METHODS FOR TOTAL VIABLE BACTERIAL CELL NUMBER

The chief cultural method for determining total viable bacterial densities has been the agar plate method. (4 - 6) Experience indicates that an approximate enumeration of total number of viable bacteria multiplying at a temperature of 35 C may yield useful information concerning the sanitary quality of the waste entering a processing or a disposal site, and provide useful information in judging the efficiency of procedures employed in solid waste processing and/or disposal operations. The viable microbial count provides valuable information concerning the microbiological quality of environmental aerosols existing in or around a waste processing plant or a disposal site.

2.1 Equipment, materials and culture media

2.1.1 Pipettes, 1.1 ml with 0.1 ml and 1 ml graduations

2.1.2 Dilution blanks, phosphate buffered solution, 99 ml ±2 ml (cold)

2.1.3 Culture dishes (100 x 15 ml) plastic, sterile

2.1.4 Water bath for tempering agar, 45±1 C

2.1.5 Incubator 35±0.5 C

2.1.6 Colony counter, Quebeck

2.1.7 Sterile glass spreader, bent rod

2.1.8 Trypticase soy agar with 7% defibrinated sheep blood (TSA+blood)

2.1.9 Tryptone glucose extract agar (TGEA)

Prepare TGE agar as indicated on label and hold in a melted condition in the water bath (45 C).

Dissolve ingredients of TSA and heat to boiling. Sterilize by autoclaving at 121 C for 15 min. Cool to 45 C and add sheep blood. Dispense in Petri plates and allow to solidify. Invert plates and place them in incubator overnight to dry.

2.2 Procedure for bacterial count by pour plate

2.2.1 Pipette 1 ml, 0.1 ml or other suitable volume of the sample into each of appropriately marked duplicate culture plates, being sure to shake each dilution bottle vigorously 25 times to resuspend material that may have settled out.

2.2.2 Add 10 to 12 ml of melted TGE agar to the sample in the Petri plate

2.2.3 Mix dilution and the agar medium by rotating or tilting the plate

2.2.4 Allow plates to solidify as rapidly as possible after pouring

2.2.5 Invert plates and incubate them at 35 C ± 0.5 C for 24 ± 2 hour.

2.2.6 Count all colonies using Quebeck colony counter, the objective being to count plates with 30 - 300 colonies

2.2.7 Compute the colony count per gm of waste (wet weight) or related solid material, per 100 ml of water. The number of bacteria should not include more than two significant figures.

2.3 Procedure for bacterial count by streak plate

2.3.1 Dispense 0.1 ml samples of the serially diluted homogenate (or liquid) on the surface of each of appropriately marked, duplicate blood agar plates containing trypticase soy agar base and 7% defibrinated sheep blood.

2.3.2 Using a sterile glass spreader and starting with the highest dilution plates, spread the inoculum evenly over the agar surface.

2.3.3 Invert plates and incubate them at 35 C for 24 hr ± 2 hr.

2.3.4 Count the number of colonies on plates with 30 to 300 colonies.

2.3.5 Select and mark colonies for further testing.

3. METHODS FOR PRESENCE OF MEMBERS OF COLIFORM GROUP

The coliform bacteria have long been used in the United States as indicators of fecal pollution in sanitary bacteriology. Some members of the coliform group organisms are found in the feces of warm-blooded animals, in the guts of cold-blooded animals, in soils, and on many plants. Studies have shown that warm-blooded animal feces from humans, animals, or birds may at any time contain disease-producing microorganisms (7). It was pointed out that cold-blooded animal feces are

quantitatively insignificant as a source of pollution, but the coliform bacteria from plants or soils that have been recently exposed to fecal pollution have the same significance as those from feces; on the other hand, the coliform bacteria deriving from soils or plants that have not been exposed to recent fecal contamination has less public health significance.

Adequate treatment of waste prior to disposal and proper operational design of a waste processing plant should remove all coliform organisms. Treated or processed waste containing coliform bacteria demonstrates an inadequate treatment and should be considered of more or less sanitary significance. The contamination of waste by fecal matter may be one avenue of transmission of pathogenic microorganisms to the environment and man.

The presence of fecal matter in waste and related materials is determined by the standard tests for the coliform group described in Standard Methods for the Examination of Water and Waste Water (4). The completed Most Probable Number (MPN) procedure is employed. The testing method includes the elevated temperature test (44.5 C) that indicates that fecal or non-fecal origin of coliform bacteria. Comparative laboratory studies conducted showed that the MPN estimate is the most suitable method for achieving a representative enumeration of the coliform organisms in solid waste and waste effluents (8).

3.1 Equipment and materials

3.1.1 Pipettes, sterile - deliveries to 10 ml, 1 ml (1.1 ml) and 0.1 ml.

3.1.2 Media prepared in fermantation tubes:

Lauryl tryptose broth

Brilliant green lactose bile broth, 2%

Lactose tryptose broth

E. C. broth

3.1.3 Media for plating:

Eosin methylene blue agar plates

Nutrient agar slants

3.1.4 Dilution blanks, phosphate buffered solution, sterile, 99 ml or 90 ml amounts.

3.1.5 Incubator - adjusted to 35 C ± 0.5 C

3.1.6 Water bath - adjusted to 44.5 C ± 0.5 C

3.2 Procedure for total coliform group

3.2.1 Presumptive test

3.2.1.1 Inoculate a predetermined volume of sample into 5 lauryl tryptose broth tubes. The portions of the sample used for inoculation should be decimal multiples and submultiples of 1 ml.

3.2.1.2 Incubate the fermentation tubes at 35 ± 0.5 C for 24 ± 2 hours.

3.2.1.3 Examine for the presence of gas. If no gas is formed incubate up to 48 ± 3 hours.

3.2.2 Confirmed test

3.2.2.1 Submit all presumptive test tubes showing any amount of gas at the end of 24 and 48 hr incubation to the confirmed test.

Using a sterile platinum loop, 3 mm in diameter, transfer one loopful of medium from the presumptive test fermentation tube to a fermentation tube containing brilliant green lactose bile broth.

3.2.2.2 Incubate the inoculated brilliant green lactose bile broth tube for 48 ± 3 hours at 35 ± 0.5 C. The presence of gas in any amount in the fermentation tubes of the brilliant green lactose bile broth within 48 ± 3 hours indicates a positive confirmed test.

3.2.3 Completed test

3.2.3.1 Submit all confirmed test tubes showing any amount of gas to the completed test. Streak an eosin methylene blue agar plate from each brilliant green bile broth tube as soon as possible after the appearance of gas.

3.2.3.2 Incubate the plates at 35 ± 0.5 C for 24 ± 2 hours.

3.2.3.3 Fish one or more typical or atypical colonies from plating medium to lactose tryptose broth fermentation tubes and nutrient agar slants.

3.2.3.4 Incubate the broth tubes and the agar slants at 35 ± 0.5 C for 24 ± 2 or 48 ± 3 hours

3.2.3.5 Prepare gram stained smears from the nutrient agar slants if gas is produced in any amount from lactose broth

3.2.3.6 Examine smears under oil immersion. If no spores are found on the slant the test may be considered "completed" and the presence of coliform organisms demonstrated.

3.3 Procedure for fecal coliform group (E. C. broth)

3.3.1 Submit all gas positive tubes from the Standard Methods presumptive test (lauryl tryptose broth) to the fecal coliform test. Inoculate a E. C. broth fermentation tube with a 3 mm loop of broth from a positive presumptive tube.

3.3.2 Incubate the broth tube in a water bath at 44.5 ± 0.5 C for 24 hours. All E.C. tubes must be placed in the water bath within 30 min. after planting.

3.3.3 Gas production in the E. C. broth fermentation tubes within 24 hours is considered a positive reaction indicating fecal origin.

3.4 Computing and Recording Most Probable Number (MPN)

The calculated estimate and the 95% confidence limits of the MPN have been presented in the current (13th) edition of Standards Methods for Examination of Water and Waste Water. (Ref. 4, Table 407, p. 673.) This table is based on five 10 ml, five 1.0 ml, and five 0.1 ml sample portions. When the series of decimal dilutions such as 1.0, 0.1, and 0.01 ml are planted, record 10 times the value in the table; if a combination of portions of 0.1, 0.01, and 0.001 ml are planted record 100 times the value in the table. MPN values for solid samples are calculated per gram of wet weight; MPN for liquid samples are recorded per 100 ml.

4. METHOD TO DETERMINE THE PRESENCE OF VIABLE HEAT-RESISTANT SPORE FORMERS

It was of importance to enumerate those heat-resistant, spore-forming microorganisms in waste, incinerator residue, quench or industrial waters which survive 80 C temperature for as long as 30 minutes. With

respect to mere survival of heat most microorganisms in an actively growing (vegetative) state are readily killed by exposures to temperatures of around 70 C for 1 to 5 minutes (9). Cells inside of solid material such as discarded meat products may escape heat longer because the heat does not penetrate immediately into the center of solid masses. Large masses of non-fluid solid matter require a long time (1-1/2 to 2 hr), even in the autoclave (121 C) to be heated thoroughly so that the center reaches a sporocidal temperature. Other reports point out (10), that although internal air temperatures of municipal incinerators usually range from 1200 to 1700 F (650 to 925 C) in continuous operation, intermittent use and overcharging of the incinerator and moisture content of the waste may interfere with sterilization of the residue.

A test of this type reveals operational problems of a waste processing plant and identifies unsatisfactory quality of waste effluents of a municipal incinerator.

4.1 Equipment and materials

4.1.1 Test tubes, sterile - screw capped, 20 x 150 mm

4.1.2 Pipettes, sterile - graduated, 10 ml

4.1.3 Water bath - electrically heated, thermostatically controlled at 80 ± 0.5 C, equipped with thermometer, range 0 to 110 C, NBS certified. Volume of water should be sufficient to absorb cooling effect of rack of tubes without drop in temperature greater than 0.5 C.

4.1.4 Test tube support - for holding tubes

4.2 Procedure

4.2.1 Transfer 10 ml from each original sample and from each successive dilution thereof to screw-capped test tubes, being careful to avoid contaminating the lip and upper portion of tube with sample.

4.2.2 Place tubes in a rack

4.2.3 Place rack of tubes in water bath at 80 C for 30 min. Tubes should be immersed so that the water line is approximately 1-1/2 inches above the level of samples in the tubes.

4.2.4 At the end of the 30 min holding period, remove the rack of tubes from the water bath and place in cold water for 5 min to cool the tubes.

4.2.5 Determine viable heat-resistant spore count by agar pour plate method (Section C, 2.2.1 - 2.2.7)

4.2.6 Report results as "viable heat-resistant spore count per gm"

5. METHODS TO DETECT ENTERIC PATHOGENIC BACTERIA

An access of fecal pollution to environment by nontreated and improperly disposed waste may add enteric pathogenic bacteria to a body of water or a water supply. The most common type of pathogen which may be found in untreated waste is Salmonella. The wide distribution of the many types of Salmonella in many species of animals with which man has contact or may use as food makes it difficult to prevent transmission to man (11). Infections may occur through food, milk, or water contaminated with infected feces or urine, or by the actual ingestion of the infected animal tissues (12). Salmonella has been found in many water supplies (13), polluted waters (17 - 19), and raw municipal refuse and in incinerator residue (14 - 20).

The detection of enteric pathogenic bacteria such as Salmonella and Shigella in municipal solid waste before and after a treatment and/or disposal determines the microbiological quality of the material and serves as a procedure to determine the efficacy of a waste treatment process in removing or destroying the fecal-borne pathogens. Results obtained in the testing may also be used for the design of epidemiological studies in other programs.

The method described below has been tested in field and has been described by Peterson and Klee (14) and Spino (20), using incubation temperatures of 39.5 C and 41.5 C.

5.1 Equipment, materials and media

5.1.1 Incubator, 37 C

5.1.2 Water baths, constant temperature, 39.5 C and 41.5 C

5.1.3 Flasks, wide mouth, 500 ml size

5.1.4 Membrane filter holder

5.1.5 Flasks, vacuum, 2000 ml size

5.1.6 Balance, with weights, 100 gm capacity

5.1.7 Needle, inoculating

5.1.8 Media and reagents

Selenite brilliant green/sulfa enrichment broth

Selenite F enrichment broth

Eosin methylene blue (EMB) agar

Salmonella - Shigella (SS) agar

Bismuth sulfite (BS) agar

McConkey's agar

Brilliant green (BG) agar

Triple sugar iron (TSI) agar

Urea medium

XLD agar

Salmonella antiserums

Shigella antiserums

Biochemical media (15)

5.1.9 Diatomaceous - earth (Johns-Manville, Celite 505), sterile

5.2 Procedure to detect pathogens in solid waste and incinerator residuc.

5.2.1 Add a previously weighed 30-gm sample to each of two flasks containing 270 ml Selenite F enrichment broth and also to each of two flasks containing 270 ml Selenite brilliant green/sulfa (SBG) enrichment broth. Shake to mix.

5.2.2 Incubate one Selenite F and one SBG flask at 39.5 C and the other two at 41.5 C for 16 to 18 hours.

5.2.3 After incubation streak one loopful from each enrichment medium on each of four plates of Salmonella-Shigella and other selective enteric media.

5.2.4 Incubate the plates at 37 C for 24 - 48 hours, and pick suspicious colonies to triple sugar iron agar slants.

5.2.5 Incubate the slants at 37 C for 24 hours and complete identification by appropriate biochemical and serological test as directed (15). Isolation and preliminary identification is described in Figure 3.

5.3 Procedure to detect pathogens in quench or industrial waters and in leachate

5.3.1 Place a portion of sterile diatomaceous - earth on the screen of a stainless steel membrane filter holder enough to form a 1" layer.

5.3.2 Filter 800 ml sample through the earth layer

5.3.3 Remove one half of the diatomaceous - earth layer with a sterile spatula and place into 90 ml of Selenite F enrichment broth; place other half of the earth layer into 90 ml of Selenite brilliant green/sulfa enrichment broth. Shake both flasks to mix.

5.3.4 Incubate both flasks in a water bath at 39.5 C for 16 to 18 hours.

5.3.5 Proceed as directed (Section C, 5.2.3. - 5.2.5)

6. METHOD FOR EXAMINATION OF STACK EFFLUENTS

6.1 As described in Methods for collection of stack effluents using the Armstrong sampler, the microorganisms are impinged into a 300 ml phosphate buffered soultion.

6.1.1 Filter 100 ml of the "inoculated" phophate buffered solution through a 0.45µ HA membrane filter (4).

6.1.2 Transfer membrane filter with sterile forceps to a culture plate containing trypticase soy agar.

6.1.3 Incubate culture plate under constant saturated humidity for 20 hours (± 2 hrs) at 35 C.

6.1.4 After incubation, remove cover from culture plate and determine colony count with the aid of a low-power (10-15 magnifications) binocular wide-field microscope. Characterize colonies using specific isolation media.

6.1.5 Remove a 10 ml portion of the "inoculated" phosphate buffered solution and examine for viable heat-resistant spores as directed (Section C, 4.2.1 - 4.2.6).

Microbioal counts are reported as organisms per 1 cu ft air. If the sample is not taken under isokinetic conditions, the results are qualitative. If the stack velocity is known and remains relatively constant, however, the rate of flow of the sampler can be adjusted to isokinetic conditions to yield quantitative results.

7. METHOD FOR EXAMINATION OF DUST

7.1 As described in Methods for collection of dust samples, the Andersen sampler is used with two types of media, trypticase soy agar (TSA-BBL product) containing 5% sheep blood and eosin methylene blue agar (EMB-Difco product). The TSA/blood agar is used to isolate a wider range of fastidious organisms such as Staphylococci, Streptococci, and Diplococci. The EMB agar is used to isolate gram-negative bacteria. The plates are incubated aerobically at 35 C for 24 hours. (Preliminary studies showed that few organisms in the dust would grow under anaerobic conditions.) Enumeration of colonies is made with a Quebec colony counter. Microbial count is reported as organisms per 1 cu ft air. At times when microbial counts are high, the sampling time is 0.25 min, thus yielding 0.25 cu ft air.

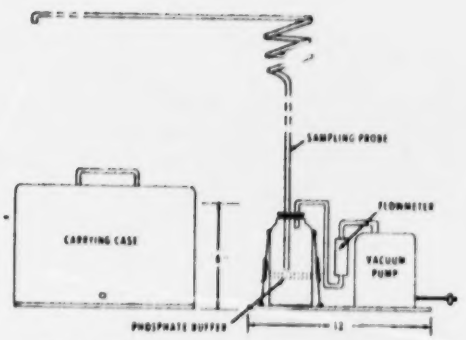


FIG. 1. *Portable sampler for microorganisms in incinerator stack emissions.*

FIGURE 2 Preparation of Decimal Dilutions

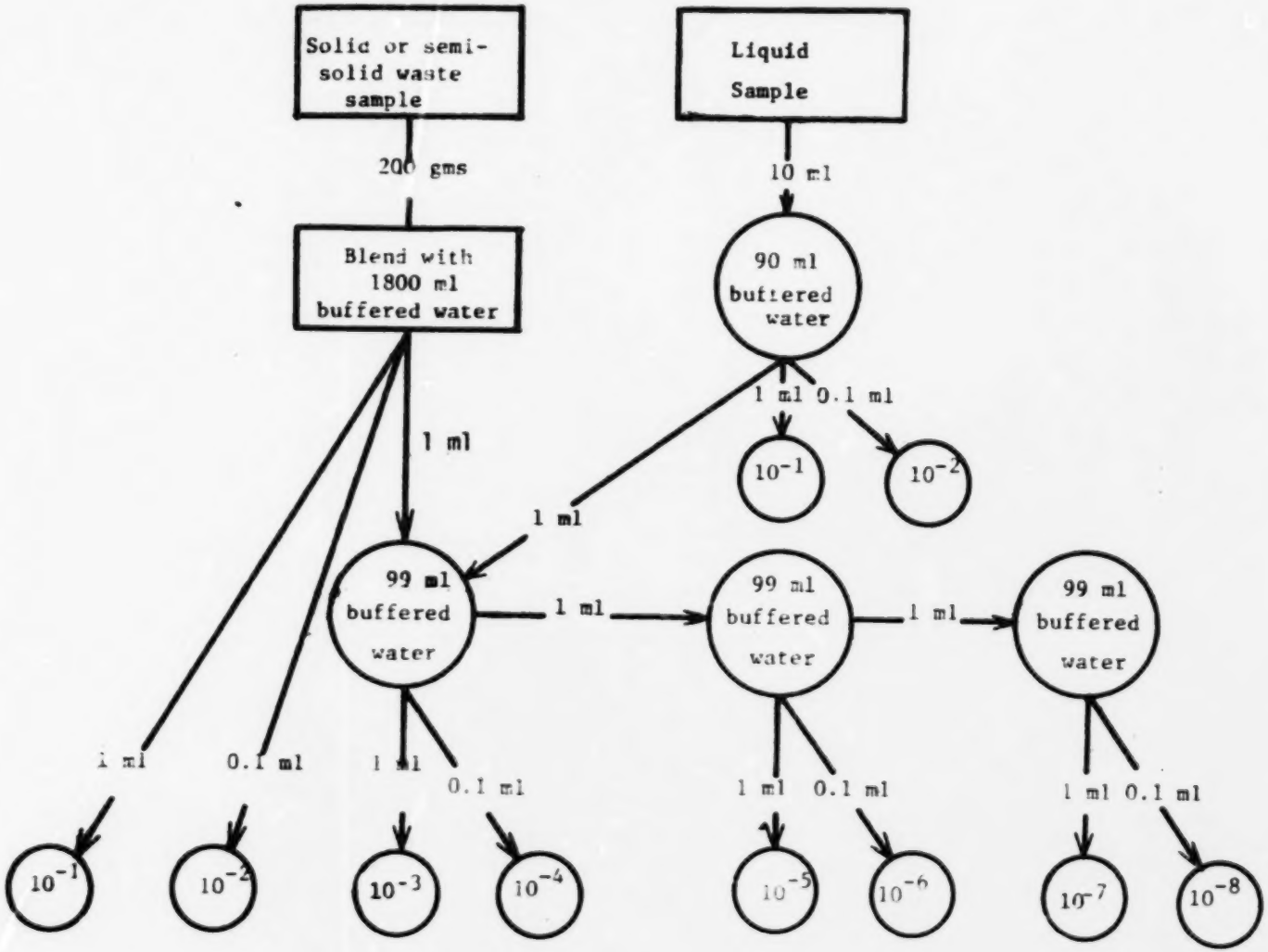
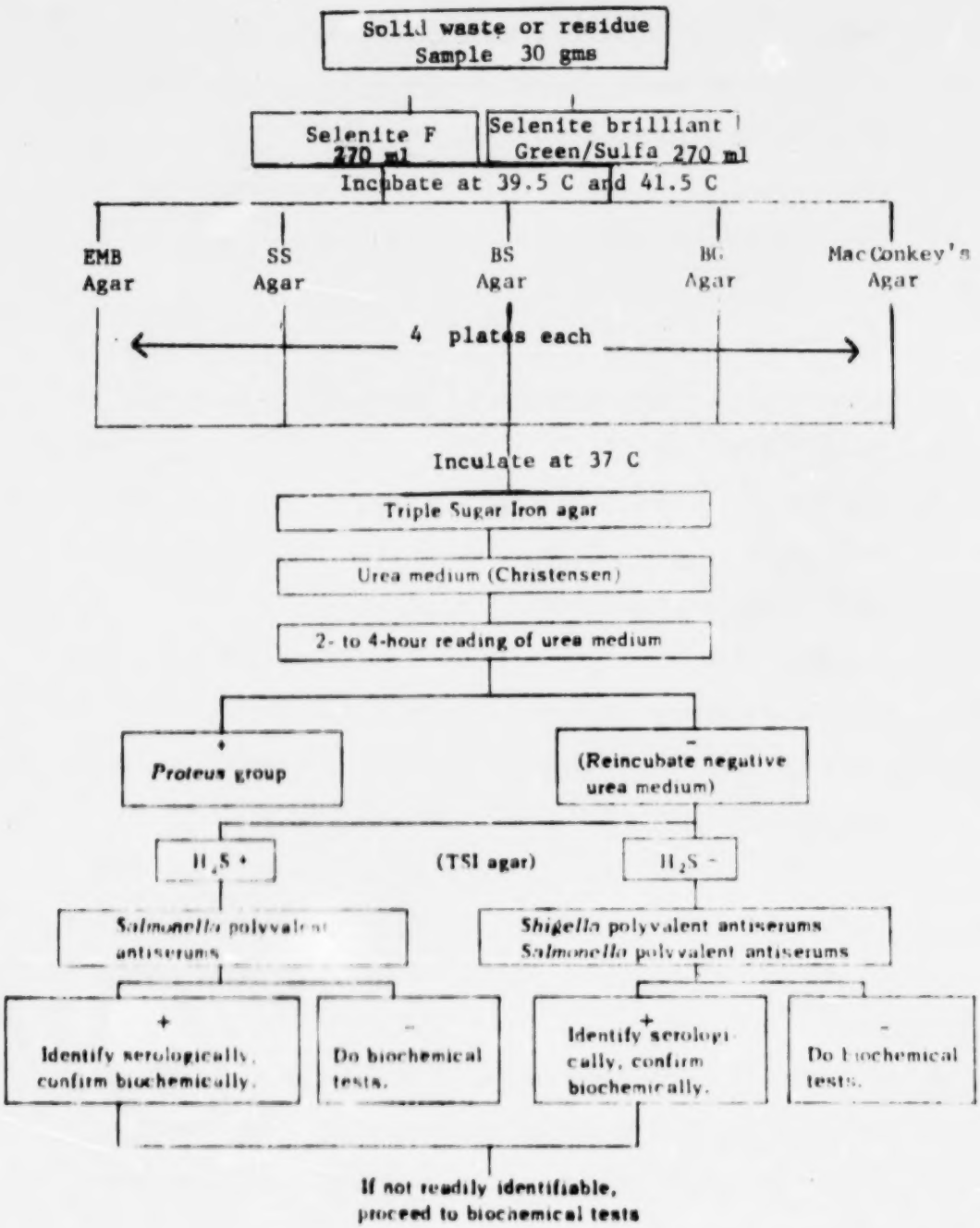


FIGURE 3 Isolation and Preliminary Identification



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